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In vitro response of strawberry cultivars and regenerants to *Colletotrichum acutatum*

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Abstract

Diseases affecting strawberry (*Fragaria* × *ananassa* Duch.) have been of major concern in recent years because of their widespread occurrence and potential for yield loss. Anthracnose, caused by the fungus *Colletotrichum acutatum*, is one of the most serious diseases of strawberry worldwide. Tissue-culture induced (somaclonal) variation provides one strategy for generating disease-resistant genotypes. As part of a program to generate strawberry germplasm resistant to anthracnose, an *in vitro* screening system was used to evaluate several commercial cultivars, Chandler, Delmarvel, Honeoye, Latestar, Pelican and Sweet Charlie propagated *in vitro*, and shoots regenerated from leaf explants of these cultivars for resistance to *C. acutatum* isolate Goff (highly virulent). Regenerants with increased levels of resistance were identified from all of the cultivars. The greatest increases in disease resistance were observed for regenerants from leaf explants of cultivars Pelican and Chandler that exhibited 17.5- and 6.2-fold increases in resistance, respectively. The highest levels of anthracnose resistance (2 to 6% leaf necrosis) were exhibited by regenerants from explants of cultivars Pelican and Sweet Charlie. These studies suggest that generating somaclonal variation may be a viable approach to obtaining strawberry plants with increased levels of anthracnose resistance.

Abbreviations: BA – benzyladenine; GA_3 – gibberellic acid; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; MS – Murashige and Skoog; PDA – potato dextrose agar; TDZ – thidiazuron

Introduction

A major constraint to strawberry production is the loss caused by diseases, and one of the most serious diseases of strawberry worldwide is anthracnose caused by the fungal species *Colletotrichum acutatum* J.H. Simmonds (Maas, 1998). *C. acutatum* causes fruit rot (Howard et al., 1992; Smith, 1998a), runner and petiole lesions, and death from crown rot (Smith, 1998b). Although fungicides are being used to control anthracnose, generating disease resistant plants is a more attractive solution to the problem because fungicides can pose a health hazard, have a negative impact on the environment, and may become unavailable due to loss of registration (Legard et al., 2002). A number of *C. acutatum* isolates have been found to exhibit resistance to the fungicide benomyl in *in vitro* assays (LaMondia, 1993; Smith and Black, 1993), and the potential exists that the pathogen will become resistant to currently used fungicides. Time and a narrow germplasm base are limiting factors in producing quality strawberry cultivars with high levels of

disease resistance via conventional breeding (Sjulin and Dale, 1987; Dale and Sjulin, 1990; Hancock and Luby, 1995). Thus, tissue culture-induced (somaclonal) variation can provide an attractive alternative strategy for generating disease-resistant cultivars since genetic variants of a cultivar can be selected *in vitro* and this approach has the potential to develop genetic variation not available in existing germplasm (Larkin and Scowcroft, 1981). Selecting for somaclonal variants has generated disease resistance in a wide range of plants, including sugarcane (*Saccharum officinarum* L.) (Krishnamurthi and Tlaskal, 1974), celery (*Apium graveolens* L.) (Heath-Pagliuso et al., 1988), tomato (*Lycopersicon esculetum* Mill.) (Barden et al., 1986), apple (*Malus x domestica* Borkh.) (Donovan et al., 1994), and peach (*Prunus persica* L. Batsch) (Hammerschlag, 1990, 1994, 2000). This approach has also generated strawberry somaclones with increased resistance to *Fusarium oxysporum* f. sp. *fragariae* (Toyoda et al., 1991) and *Alternaria alternata* (Takahashi et al., 1993). Previously, we reported briefly on an *in vitro* screening system to evaluate strawberry cultivars and somaclones for resistance to *C. acutatum* (Garcés et al., 2000; Garcés et al., 2002). Building on our previous work, the objectives of the current research were to determine if *in vitro* screening of strawberry plants for anthracnose resistance produced similar results to that reported *ex vitro*, and to determine if leaf-derived regenerants from susceptible as well as resistant cultivars demonstrate variation in resistance against *C. acutatum*.

Materials and methods

Plant material

Actively growing, virus-indexed shoot cultures from three anthracnose fruit- or runner-susceptible strawberry cultivars [Latestar (G.J. Galletta, personal communication), Chandler (Smith et al., 1998), Honeoye (Denoyes-Rothan et al., 1999)], and three anthracnose fruit- or runner-resistant cultivars [Delmarvel (Galletta et al., 1995), Sweet Charlie (Chandler et al., 1997), and Pelican (Smith et al., 1998)] were obtained from Dr. G. Galletta, USDA/ARS, Fruit Laboratory, Beltsville, MD. These shoots were maintained on shoot proliferation medium containing MS salts (Murashige and Skoog, 1962), nicotinic acid (50 mg l^{-1}), pyridoxine-HCl (50 mg l^{-1}), glycine (200 mg l^{-1}), thiamine-HCl (10 mg l^{-1}), myo-inositol (1 mg l^{-1}), 5.7 μM IAA, 4.4 μM BA, 0.03 μM GA_3, 87.6 mM sucrose and 0.8% (w/v) Bacto agar. The pH was adjusted to 5.8 before autoclaving at 121 °C, 131 kPa for 15 min. Filter sterilized IAA was added to the autoclaved medium. The medium was then dispensed into glass jars (150 ml per jar) that were sealed with plastic wrap. Shoots were incubated at 25 °C under a 16-h photoperiod provided by cool white fluorescent lights at 39 μmol m^{-2} s^{-1} and sub-cultured every 4–5 weeks.

Shoot regeneration from parental cultivars

The basal half of leaflets from shoot cultures (of parental cultivars maintained on shoot propagation medium for 6 months to 3 years), placed on fresh propagation medium for two weeks, was positioned abaxial side up in petri dishes containing strawberry regeneration medium (35 ml per petri dish). The regeneration medium was composed of MS salts, thiamine-HCl (0.4 mg l^{-1}), myo-inositol (100 mg l^{-1}), casein hydrolysate (600 mg l^{-1}), 1 or 10 μM thidiazuron (TDZ), 49 μM IBA, 87.6 mM sucrose and 0.8% (w/v) Bacto agar. Explants were incubated in the dark at 25 °C for 2 weeks, and then incubated under a 16-h photoperiod as indicated above. Explants were subcultured every 2 weeks for 6 weeks, placed on the same medium without growth regulators for 2 weeks, and then adventitious shoot regenerants were transferred to shoot proliferation medium as mentioned above to produce a minimum of 60 shoots per somaclone.

Preparation of inoculum

Colletotrichum acutatum isolate Goff was initiated from silica gel cultures maintained at USDA/ARS in Poplarville, Miss. (Smith and Black, 1990). Cultures were maintained on Difco PDA at 24 °C, under cool white fluorescent lights at 44 μmol m^{-2} s^{-1}. Cultures were transferred each week by transferring spores in an X pattern over PDA medium with a sterile loop. Spores from a one-week-old culture of *C. acutatum* isolate Goff were diluted with sterile, double distilled water to obtain a concentration of $1–2 \times 10^6$ spores ml^{-1}, determined with a hemacytometer. The spore

suspension (20 ml) was poured into sterile plastic petri dishes (100 mm × 25 mm).

Inoculation and evaluation

Prior to inoculation, shoots from parental cultivars (maintained *in vitro* on shoot propagation medium for 6 months to 3 years) and shoot regenerants (regenerated from leaflets without a callus phase and maintained *in vitro* on shoot propagation medium for 6 months to 2 years) were transferred to shoot elongation medium containing NH_4NO_3 (1.65 g l^{-1}), $Ca(NO_3)_2 \cdot 4H_2O$ (10 g l^{-1}), KNO_3 (2.5 g l^{-1}), KH_2PO_4 (2.5 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (2.5 g l^{-1}), 100 μM each of NA_2-EDTA and $FeSO_4 \cdot 7H_2O$, MS micro salts and vitamins with the exception of myo-inositol (1 mg l^{-1}), and 0.2 M glucose, pH 5.8. This medium was autoclaved and dispensed as above for the proliferation medium. Shoots were incubated as described above.

One fully expanded leaf (1.5–2.5 cm in diameter) was removed from each shoot that was maintained on shoot elongation medium for 4 weeks. Leaves were incubated at 20 °C for 24 h in a spore suspension of *C. acutatum* or in sterile distilled water (during evaluation of cultivars), and then sub-cultured onto 0.5% Difco Bacto agar in petri dishes (100 mm × 15 mm), (five leaves per petri dish, three petri dishes per replication, and four replications over time). Percentage of necrosis was scored after 7 days (Figure 1). As a check for virulence of the pathogen, susceptible cultivar Latestar or a consistently susceptible regenerant was inoculated along with the regenerants.

Data analysis

To determine if differences between the six genotypes could be detected with this assay, an analysis of variance was conducted. Additional analyses of variance were done to determine if differences could be detected between a cultivar and the somaclonal variants derived from it. In all cases, an analysis of means was done: the 15 necrosis scores were averaged within each of the four replicate evaluations of each genotype. The analysis of variance was performed on the four replication means for each genotype using genotype and replication as sources of variance. The command used was "proc glm" in SAS 8.2 (SAS Institute Inc., 1999). The experimental errors derived from each analysis were used to calculate the least significant difference value (LSD) that was used to identify genotypes with significantly different mean necrosis scores ($p \leq 0.05$).

Figure 1. No necrosis (right) and 35% necrosis (left), respectively, on leaves of *in vitro* cultivar Honeoye, 7 days after incubating leaves for 24 h in either sterile distilled water or a spore suspension of *Colletotrichum acutatum* isolate Goff and then placing leaves on Difco Bacto agar.

Results and discussion

Inoculation evaluations for strawberry cultivars

Except for cultivar Delmarvel, the severity of infection (% leaf necrosis) on the cultivars, following *in vitro* inoculation with *C. acutatum* (Figure 2), corresponded to the fruit susceptibility classification under field conditions (Chandler et al., 1997; Smith et al., 1998; Denoyes-Rothan et al., 1999; Galletta, personal communication). Although 'Delmarvel' was evaluated previously as a resistant cultivar (Galletta et al., 1995), our results clearly show 'Delmarvel' with 86% leaf necrosis (Figure 2, Table 1), to be susceptible to *C. acutatum*. Differences between *in vitro* and *ex vitro* conditions could account for the differences in response to *C. acutatum*. Such differences have been reported for strawberry in response to *Phytophthora cactorum* (Rosati et al., 1989; Sowik et al., 2001) and for apple in response to *Erwinia amylovora* (Visuer and Tapia y Figueroa, 1987), thus making it unacceptable to use an *in vitro* screening approach to evaluate that germplasm. Another explanation for the susceptibility of 'Delmarvel' *in vitro* could have been due to our using the highly virulent isolate Goff (Smith and Black, 1990; Denoyes and Baudry, 1995) compared with the *C. acutatum* used by Galletta et al. (1995). Smith (Smith, unpublished data) noted that 'Delmarvel' was susceptible to *C. acutatum* isolate Goff, and several studies (Smith and Black, 1990; Denoyes and Baudry, 1995) have indicated

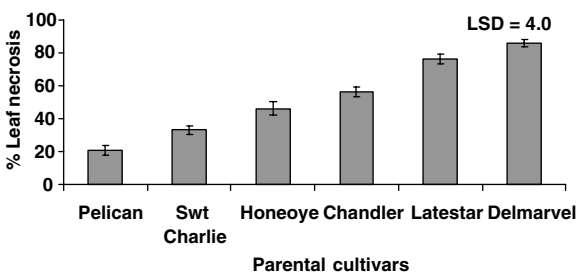

Figure 2. Percentage of infection on leaves of strawberry cultivars Chandler, Delmarvel, Honeoye, Latestar, Pelican and Sweet Charlie (Swt Charlie) following inoculation *in vitro* with *Colletotrichum acutatum* Goff. Error bars represent the standard error for each cultivar. Differences among cultivar leaf necrosis means across four replications were determined using the Least Significant Difference value, (LSD), $p \leq 0.05$, for each comparison.

that cultivars can vary significantly in disease susceptibility according to the isolate used. Taking the above into consideration, our results suggest that our *in vitro* assay with isolate Goff can be used to screen for anthracnose resistance in strawberry; thus, this same assay was used to screen strawberry regenerants from leaf explants of these same cultivars to this same pathogen. Sowik et al. (2001) suggested that *in vitro* screening of strawberry could be useful for identifying somaclonal variants tolerant to infection with *Verticillium dahliae*, since they observed that the reaction of strawberry cultivars *in vitro* was similar to their sensitivity to Verticillium wilt in the field. However, this is in contrast to Gaggioli et al. (1989) who were unsuccessful in developing an *in vitro* assay to screen for resistance to *V. dahliae*.

Inoculation evaluations for strawberry adventitious shoot regenerants

Regenerants with significantly higher levels of resistance to *C. acutatum* Goff compared with the parent cultivar were observed for all cultivars (Table 1). These results suggest that it is possible to screen at the whole plant level *in vitro* for somaclonal variants with increased levels of resistance to *C. acutatum*. Although *in vitro* selection at the cellular level is often recommended to obtain disease resistance because very large populations of cells ($>10^6$) can be screened at one time, this approach can only be used if there is a selective agent available that is involved in disease development and acts at the cellular level (Hammerschlag, 1992). Regenerating plants and screening at the whole plant level is a much simpler approach; however, only a limited numbers of plants can be evaluated. This latter approach has been used successfully to obtain heritable disease resistance in maize (*Zea mays* L.) (Brettell and Thomas, 1980), sugarcane (Krishnamurthi and Tlaskal, 1974) and tomato (Barden et al., 1986). In all cases, populations of <500 were screened. Isolation of mutants from plant tissue cultures without applying any selection pressure can be achieved when small populations are used because growing cells *in vitro* yields a high frequency of somaclonal variants that can express agriculturally useful traits (Larkin and Scowcroft, 1981). The frequency of genetic variation has been reported to be as high as 30% to 40% for the number of plants showing

Table 1. Percentage of leaf necrosis on leaves of strawberry regenerants and parental cultivars Chandler, Delmarvel, Honeoye, Latestar, Pelican, and Sweet Charlie (SWCH) following inoculation *in vitro* with *Colletotrichum acutatum* Goff[a]

Genotype	Necrosis	Genotype	Necrosis	Genotype	Necrosis	Genotype	Necrosis	Genotype	Necrosis	Genotype	Necrosis
C9C-2	9	D7C-27	37	H9C-13	25	P9C-31	2	L9C-26	46	SC9C-20	6
C9C-14	10	D9C-38	42	H9C-3	29	P9C-18	2	L7C-2	48	SC7C-2	9
C7C-1	13	D7C-28	50	H9C-2	35	P9C-20	2	L9C-25	52	SC9C-27	9
C7C-2	17	D9C-21	55	H9C-1	41	P9C-33	3	L9C-28	55	SC9C-12	10
C7C-10	17	D7C-11	55	H9C-4	41	P9C-24	3	L7C-1	59	SC9C-13	11
C7C-8	17	D7C-5	56	Honeoye	46	P9C-28	3	L9C-23	60	SC9C-7	15
C9C-22	18	D9C-14	56	H04–5	55	P9C-21	4	L9C-17	62	SC9C-22	16
C9C-30	19	D9C-9	57	H03–3	55	P9C-30	5	L9C-14	63	SC9C-11	17
C9C-1	21	D7C-8	57	H03–6	58	P9C-23	5	L9C-12	64	SC9C-28	19
C9C-4	21	D9C-29	59	H9C-12	60	P9C-32	5	L9C-16	67	SC7C-1	21
C7C-4	23	D7C-12	60	H9C-11	60	P9C-22	6	L9C-18	69	SC9C-17	22
C7C-5	23	D9C-13	62	H04–4	62	P9C-19	11	L9C-29	69	SC9C-14	22
C9C-11	26	D9C-18	62	H03–5	69	P9C-25	14	L9C-11	70	SC9C-5	27
C7C-3	28	D9C-17	63	H9C-9	70	P9C-6	18	L9C-24	71	SC9C-4	29
C9C-12	29	D9C-32	64	H04–3	72	Pelican	21	L9C-13	76	SC9C-25	30
C9C-16	31	D9C-37	67	H04–7	73	P9C-27	22	Latestar	76	SC9C-21	31
C9C-19	32	D7C-19	67	H9C-6	74	P9C-12	23			SC9C-9	31
C9C-5	34	D7C-25	69	H9C-5	75	P9C-1	28			SC9C-1	32
C9C-20	35	D9C-20	73	H9C-8	78	P9C-2	30			SWCH	33
C9C-3	36	Delmarvel	86	H03-1	79	P9C-3	33			SC9C-6	35
Chandler	56			H01-1	83	P9C-17	35				
				H9C-7	85						
LSD	2.9		4.1		3.3		1.9		3.7		2.8

[a] Differences in leaf necrosis means between regenerants and the cultivar from which they were derived were determined using the Least Significant Difference value (LSD), $p < 0.05$. Each leaf necrosis mean was derived from four replications of 15 samples per replication.

some type of variation, and from 0.2% to almost 3% for variation in a single trait (Lorz and Scowcroft, 1983; Zong-xiu et al., 1983; Evans et al., 1984; Irvine, 1984). Hammerschlag (1990) reported that 33–100% of peach regenerants (maintained under greenhouse conditions, but leaves assayed *in vitro*) were either significantly more resistant or susceptible to *Xanthomonas campestris* pv. *pruni* compared with the parent cultivar; however, only one regenerant retained increased levels of disease resistance under field conditions and had heritable resistance (Hammerschlag et al., 1994; Hammerschlag, 2000). In the present study, 15–21 regenerants per cultivar were evaluated, and the percentage of somaclonal variants with significantly higher levels of resistance to *C. acutatum* ranged from 25% for cultivar Honeoye to 100% for cultivars Chandler and Delmarvel (Table 1). In comparison to work on peach where leaves for the assay were obtained from regenerants that were maintained in the greenhouse (Hammerschlag et al., 1990), in the present study, leaves were obtained from regenerants maintained *in vitro*, which may explain the high level of variation observed. It remains to be determined whether any of the strawberry anthracnose variation is heritable. Toyoda et al. (1991) reported that two *Fusarium* resistant lines were obtained from a total of 1225 regenerants of strawberry; however, although resistance held up through propagation, heritability has yet to be determined. Field resistance, but not heritability, has also been reported for strawberry somaclones in response to several isolates of *Alternaria alternata* (Takasashi et al., 1993). The differences among cultivars in the frequency of variation in response to *C. acutatum* suggests that somaclonal variation is genotype-dependent. Other studies have also shown that the genotype of the plant can affect the amount of variability that occurs as a consequence of culturing tissues *in vitro* (McCoy et al., 1982; Lorz, 1984). Thus, this study suggests

that if somaclonal variation in strawberry is a desired outcome, explants with different genetic backgrounds should be used.

The greatest increases in disease resistance were observed for somaclones of cultivars Pelican and Chandler that exhibited 17.5- and 6.2-fold increases in resistance, respectively, whereas the highest levels of anthracnose resistance (2–6% leaf necrosis) were exhibited by somaclones of cultivars Pelican and Sweet Charlie (Table 1), already the most resistant in the field. These studies suggest that increased levels of resistance can be generated from a susceptible cultivar (e.g., Chandler) by using tissue culture techniques, but to obtain the highest levels of resistance, it is best to start with germplasm already exhibiting some degree of resistance (e.g., Pelican, Sweet Charlie). These same observations were reported for peach in response to *X. campestris* pv. *pruni* (Hammerschlag et al., 1994).

In conclusion, this study provides some evidence that although tissue culture of strawberry may not be either a faster or easier method than breeding for developing resistance to *C. acutatum*, it may provide an alternate and effective means of obtaining high levels of resistance and of obtaining resistance in susceptible germplasm that may otherwise be unattainable. Yet to be determined, and critical to establishing this as a definitive approach, is to determine the stability (under field conditions) and heritability of this anthracnose resistance, and whether other traits besides disease resistance have been affected.

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References

Barden KA, Smith SS & Murakishi HH (1986) Regeneration and screening of tomato somaclones for resistance to tobacco mosaic virus. Plant Sci. 45: 209–213

Brettell RIS & Thomas E (1980) Reversion of Texas male-sterile cytoplasm maize in culture to give fertile T-toxin resistant plants. Theor. Appl. Genet. 58: 55–58

Chandler DK, Albregts EE & Howard CM (1997) 'Sweet Charlie' strawberry. HortScience 32: 1132–1133

Dale A & Sjulin TM (1990) Few cytoplasms contribute to North American strawberry cultivars. HortScience 25: 1341–1342

Denoyes B & Baudry A (1995) Species identification and pathogenicity study of French *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. Phytopathology 85: 53–57

Denoyes-Rothan B, Lafargue M & Guerin G (1999) Fruit resistance to *Colletotrichum acutatum* in strawberries. Plant Dis. 83: 549–553

Donovan AM, Morgan R, Valobra-Piagnani C, Ridout MS, James DJ & Garrett CM (1994) Assessment of somaclonal variation in apple. I. Resistance to the fire blight pathogen Erwinia amylovora. J. Hort. Sci. 69: 105–113

Evans DA, Sharp WR & Medina-Filho HP (1984) Somaclonal and gametoclonal variation. Am. J. Bot. 71: 759–774

Gaggioli D, Maas JL, Messetti B & Rosati P (1989) The use of culture of the fungus to screen for *Verticillium albo-atrum* in strawberry. Acta Hort. 265: 61–68

Galletta GJ, Maas JL, Enns JM, Draper AD, Fiola JA, Scheerens JC, Archbold DD & Ballington JR Jr (1995) 'Delmarvel' strawberry. HortScience 30(5): 1099–1103

Garcés S, Koch-Dean M, Maas J, Smith BJ & Hammerschlag F (2000) Screening strawberry *in vitro* for response to *Colletotrichum acutatum*. HortScience 35: 445

Garcés S, Hammerschlag F, Maas JL, Koch-Dean M & Smith B (2002)Increased resistance to *Colletotrichum acutatum* is exhibited by leaf explant regenerants derived from several strawberry cultivars. In: Hokanson SC & Jamieson AR (eds) Strawberry Research to 2001; Proceedings 5th North American Strawberry Conference. ASHS Press, Alexandria, VA (pp 54–57)

Hammerschlag FA (1990) Resistance responses of plants regenerated from peach callus cultures to *Xanthomonas campestris* pv. *pruni*. J. Am. Soc. Hort. Sci. 115: 1034–1037

Hammerschlag FA (2000) Resistant responses of peach somaclone 122–1 to *Xanthomonas campestris* pv. *pruni* and to *Pseudomonas syringae* pv. *syringae*. HortScience 35: 141–143

Hammerschlag FA, Werner DJ & Ritchie DF (1994) Stability of bacterial leaf spot resistance in peach regenerants under *in vitro*, greenhouse and field conditions. Euphytica 76: 101–106

Hancock JF & Luby JJ (1995) Adaptive zones and ancestry of the most important North American strawberry cultivars. Fruit Var. J. 49: 85–89

Heath-Pagliuso S, Pullman J & Rappaport L (1988) Somaclonal variation in celery: Screening for resistance to *Fusarium oxysporum* f. sp. *apii*, race 2. Theor. Appl. Genet. 75: 446–451

Howard CM, Maas JL, Chandler CK & Albregts EE (1992) Anthracnose of strawberry caused by the *Colletotrichum* complex in Florida. Plant Dis. 76: 976–981

Irvine JE (1984) The frequency of marker changes in sugarcane plants regenerated from callus culture. Plant Cell Tiss. Org. Cult. 3: 201–209

Krishnamurthi M & Tlaskal J (1974) Fiji disease resistant *Saccharum officinarum* var. Pindar subclones from tissue cultures. Proc. Intl. Soc. Sugarcane Technol. 15: 130–137

LaMondia JA (1993) *In vitro* evaluation of fungicides against *Colletotrichum acutatum* isolates from strawberry. Adv. Strawberry Res. 12: 34–37

Larkin PJ & Scowcroft WR (1981) Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197–214

Legard DE, Mertely JC, Chandler CK, Price JF & Duval JD (2002) Management of strawberry diseases in the 21st century: chemical and cultural control of Botrytis fruit rot. In: Hokanson SC & Jamieson AR (eds) Strawberry Research to 2001; Proceedings 5th North American Strawberry Conference. ASHS Press, Alexandria, VA (pp 58–63)

Lorz H (1984)Variability in tissue culture derived plants. In: Arber W (ed) Genetic Manipulation; Impact on Man and Society. Cambridge University Press, Cambridge (pp 103–114)

Lorz H & Scowcroft WR (1983) Variability among plants and their progeny regenerated from protoplasts of Su/su heterozygotes of *Nicotiana tabacum*. Theor. Appl. Genet. 66: 67–75

Maas JL (ed) (1998) Compendium of Strawberry Diseases. APS Press, St. Paul, MN

McCoy TJ, Phillips RL & Rines HW (1982) Cytogenetic analysis of plants regenerated from oat (*Avena sativa*) tissue cultures; high frequency of partial chromosome loss. Can. J. Genet. Cytol. 24: 37–50

Murashige T & Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15: 473–479

Rosati P, Messetti B, Gagioli D & Lovato A (1989) Screening for *Phytophthora cactorum* resistance with culture filtrates of the fungus. Acta Hort. 265: 123–128

SAS Institute Inc. (1999) SAS Online Doc® Vol 8. SAS Institute Inc, NC

Sjulin TM & Dale A (1987) Genetic diversity of North American strawberry cultivars. J. Amer. Soc. Hort. Sci. 112: 375–385

Smith BJ (1998a)Anthracnose fruit rot. In: Maas JL (ed) Compendium of Strawberry Diseases, 2nd Edn. APS Press, St. Paul, MN (pp 31–33)

Smith BJ (1998b)Anthracnose crown rot. In: Maas JL (ed) Compendium of Strawberry diseases, 2nd Edn. St. Paul, MN, APS Press (pp 46–48)

Smith BJ & Black LL (1990) Morphological, cultural and pathogenic variation among *Colletotrichum* species isolated from strawberry. Plant Dis. 74: 69–76

Smith BJ & Black LL (1993) *In vitro* fungicide studies show the occurrence of benomyl-resistant *Colletotrichum* spp. from strawberry. Adv. Strawberry Res. 12: 42–48

Smith BJ, Gupton CL, Galletta GJ, Maas JL, Enns JM, Ballington JR Jr, Constantin RJ, DiVittorio TJ & Himelrick D (1998) 'Pelican' strawberry. HortScience 33(6): 1082–1084

Sowik I, Bielenin A & Michalczuk L (2001) *In vitro* testing of strawberry resistance to *Verticillium dahliae* and *Phytophthora cactorum*. Scientia Hort. 88: 31–40

Takahashi H, Matsumoto T & Takai T (1993) Somaclonal variants from strawberry cv. Morioka-16, M16-AR1, 2 and 3 resistant to several isolates of *Alternaria alternata* strawberry pathotype occurring in Tohoku and Hokkaido. J. Jpn. Soc. Hort. Sci. 61: 821–826

Toyodo H, Horikoshi K, Yamano Y & Oochi S (1991) Selection for *Fusarium* wilt disease resistance from regenerants derived from leaf callus of strawberry. Plant Cell Rep. 10: 167–170

Viseur MJ & Tapia y Figueroa M (1987) *In vitro* co-culture as a tool for the evaluation of blight resistance in pears and apples. Acta Hort. 217: 273–282

Zong-xiu S, Cheng-zhang Z, Kangle S, Xiu-fang Q & Yaping F (1983) Somaclonal genetics of rice, *Oryza sativa* L. Theor. Appl. Genet. 67: 67–73